

Qingjie Fuzheng Granules treat ulcerative colitis via regulating Th17/Treg cell balance

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Abstract

Objective:

To investigate the anti-inflammatory properties of Qingjie Fuzheng Granules (QFG) *in vivo* experiments using a DSS-induced ulcerative colitis (UC) model, and to elucidate the mechanism by which QFG alleviates UC by examining the Th17/Treg cell balance.

Methods: The DSS-induced UC mouse model was established, and the mice were administrated with QFG (1 g/kg) or saline by gavage. The general growth of the mice, including body weight, fecal occult blood, and disease activity index (DAI), was observed, and the length of the colon was recorded. HE staining was utilized to examine the pathological injury of the colon tissue. The expression levels of TGF- β , IFN- γ , IDO1, IL-1 β , TNF- α , IL-6, IL-17, IL-21, IL-22, IL-25, IL-10 in serum were detected by ELISA or Bio-Plex. The relative mRNA expressions in spleen and colon tissues were detected by RT-qPCR. The protein expressions of ROR γ t, Foxp3 or IDO1 in spleen and colon were detected by Western Blot or Immunohistochemical.

Results:

QFG demonstrated potential for improving the overall pathological conditions of UC mice induced by DSS, as evidenced by its significant inhibition of colon length shortening and improvement of colon tissue pathology. Additionally, QFG exhibited the ability to decrease the expression of pro-inflammatory cytokines IL-1 β , TNF- α , IFN- γ and IL-6, as well as IDO1 expression. Moreover, QFG significantly reduced the expression of Th17-related cytokines (IL-17, IL-21, IL-22, IL-25) and concurrently increased the expression of Treg-related cytokines (IL-10, TGF- β). The expression of transcription factor ROR γ t was observed to decrease while the transcription factor Foxp3 was observed to increase in colon and spleen.

Conclusion:

QFG has demonstrated the ability to suppress inflammation in mice with DSS-induced UC. This effect is achieved through the inhibition of Th17 cell differentiation, the promotion of Treg cell differentiation, and the maintenance of Th17/Treg cell balance. These actions are mediated by the regulation of transcription factors ROR γ t and Foxp3. This mechanism may contribute significantly to the observed inhibition of colon inflammation in mice treated with QFG.

1. Introduction

Ulcerative colitis (UC) is a chronic non-specific inflammatory bowel disease, characterized by weight loss, fatigue, abdominal pain, increased defecation, bloody mucus stool, and other symptoms. Studies have shown that the risk of Colitis-Associated Colon Cancer (CACC) is nearly 2–3 times higher among UC patients compared with the general population ^[1]. Therefore, it is urgent to find effective treatments for UC.

Among the many pathogenic factors leading to UC, the intestinal mucosal immune system plays an important role in it. CD4⁺ T cells are an essential component of the intestinal mucosal immune system. Helper T cells 17 (Th17) and Regulatory T cells (Treg) are two subsets of CD4⁺ T cells differentiated under different conditions [2]. In healthy intestinal tract, Th17 cells can protect the body from infection, but during inflammation, cytokines such as interleukin IL-17, IL-21, IL-22 and IL-25 produced by Th17 cells can lead to recruitment and activation of inflammatory cells [3], induce secretion of other cytokines and chemokines, and aggravate mucosal inflammation [4, 5]. Retinoic acid-associated orphan receptor γ t (ROR γ t) is a specific transcription factor expressed by Th17 cells, which plays an important regulatory role in the initial differentiation and maturation of Th17 cells. Studies have revealed that ROR γ t effectively maintains the pathogenicity of Th17 cells by inhibiting the production of IL-10, further amplifying intestinal inflammation [6]. Knocking down ROR γ t can inhibit the pathogenicity of mature Th17 cells, promote their transformation into Th2 cells, and stimulate the secretion of IL-4 and other anti-inflammatory factors [7]. ROR γ t has become an important drug target for regulating intestinal mucosal inflammation. Treg cells, a distinct subset of T cells, are primarily controlled by Forked head/wing helix transcription factor 3 (Foxp3). Foxp3 serves as the specific transcription factor for Treg cells, it controls the development of Treg cells and induces and maintains the anti-inflammatory properties of Treg cells [8]. Additionally, Foxp3 plays a role in suppressing both adaptive and innate immune responses. Initial CD4⁺ T cells can be prompted to undergo differentiation into Treg cells when exposed to TGF- β , while the presence of both TGF- β and IL-6 leads to the differentiation of Th17 cells. The interrelation between Th17 and Treg cells can be observed in their differentiation process and reciprocal inhibition in functions, collectively contributing to the homeostasis of the immune microenvironment. Th17/Treg Imbalance can potentially contribute to the development of various autoimmune diseases, including UC, which may be one of the important reasons for the incidence of UC [9, 10].

Qingjie Fuzheng Granules (QFG) is a traditional Chinese medicine compound. It is formulated of four herbs, including *Hedyotis diffusa* Willd, Malt, *Astragalus*, and *Scutellaria barbata* D. Don. [11] Previous experimental studies have shown that QFG possesses the ability to impede the proliferation, migration and lymphangiogenesis of colorectal cancer cells, induces the apoptosis of these cells [12–15], and alleviates intestinal damage caused by 5-FU [16]. It can also repair intestinal mucosal barrier, inhibit intestinal fibrosis, rectify the imbalance of intestinal flora, and inhibit the occurrence and development of intestinal inflammation induced by Dextra sulfate sodium (DSS) in mice with UC. However, the mechanism through which QFG alleviates symptoms of UC by modulating the balance of Th17/Treg and suppressing intestinal inflammation remains uncertain.

This study aims to establish a UC model in vivo, in order to explore the inhibition of QFG on colon inflammation induced by DSS in UC mice, and reveal the mechanism of QFG inhibition on UC development by examining the balance between Th17 cells and Treg cells. The findings of this study will contribute to the theoretical and experimental foundation for the potential clinical use of QFG in the treatment of UC.

2. Materials and methods

2.1 Preparation of QFG

QFG was purchased from the Affiliated People's Hospital of Fujian University of Traditional Chinese Medicine (Fuzhou, Fujian, China). It was dissolved into 125 mg/mL solution with saline, ultrasound was carried out for about 30 min, then the drug was filtered with a 0.45 µm filter, the dose for mice was 1g/kg.

2.2 Animal models and treatment

Balb/c mice were purchased from SLAC Laboratory Animal Technology Co., Ltd. (Shanghai, China). The mice were randomly divided into three groups according to their body weight: Control group, Model group and QFG group, with 10 mice in each group. Except for the Control group, the mice in other groups were administered drinking water containing 3.5% DSS (216011090, MP, USA) for 7 days. Following the ingestion of the 3.5% DSS solution from the second day onwards, the mice showed weight loss, fecal occult blood, diarrhea and other symptoms, which were consistent with the findings reported in the existing literature^[17].

At present, the mice were experiencing colitis. Mice in the QFG group were orally given QFG at a dosage of 100µL/10g per day. Meanwhile, mice in the Control group and Model group were given an equivalent volume of saline for 7 days. On day 15 of the experiment, the colon tissue was taken from the cecum to the end of the proximal anus, and the length of the colon was measured. According to the formula: Disease activity index (DAI) = mouse weight loss score + fecal occult blood score + diarrhea score (Table S1-3). Immunohistochemistry (IHC), reverse transcription quantitative polymerase chain reaction (RT-qPCR), and western blot analyses were performed on both spleen and colon tissues.

2.3 HE staining

First, the embedded wax tissue was sectioned into 4 µm slices and subjected to baking at 60 °C for 2 h. Then the tissue was dewaxed and rehydrated. Following these steps, hematoxylin staining (G1140, Solaibio, Beijing, China) and eosin staining (G1100, Solaibio, Beijing, China) were performed. Finally, the tissue was air-dried and sealed with neutral resin, and histopathological conditions were observed under an inverted microscope (Leica, Solms, Germany). Random fields from each section were captured at magnifications of 100× and 200×.

2.4 IHC Assays

IHC assays were performed as described previously. In a brief, 4 µm slices from Paraffin-embedded tissues were treated with primary antibodies IDO1 ((66528-1-Ig), Protein Tech Group, 1:300, Wuhan, China); (RORC (ab207082), Foxp3 (ab215206), Abcam, 1:300, USA) overnight at 4°C. Subsequently, all sections were subjected to a 30-minute incubation with an appropriate secondary antibody, followed by a 10-minute treatment with streptomycin anti-bioprotein-peroxidase (KIT-9710, MXB Biotechnologies) at

room temperature. Finally, the sections were stained using a DAB kit (DAB-0031, MXB Biotechnologies) and hematoxylin. Positive cells that express brown were observed and documented ($\times 200$), ($\times 400$) by a microscope (Leica, Solms, Germany). The quantification of IHC assays was evaluated by calculating the ratio of positive cell number to the total cell number in six fields.

2.5 Cytokines assay

The levels of IFN- γ , TGF- β , IDO1 were detected using Enzyme-linked immuno sorbent assay (ELISA) according to the manufacturers' instructions (Jiangsu Meimian Industrial Co., Ltd.). And the levels of IL-1 β , TNF- α , IL-6 and Th17-related cytokines (IL-17, IL-21, IL-22, IL-25) and Treg-related cytokines (IL-10, TGF- β) were measured using Bio-plex assay according to the manufacturers' instructions (Bio-plex (12010828, CA, USA)).

2.6 Western blot assay

The total proteins of the intestinal tissue were extracted with a protein extraction kit. The lysates were loaded on a 10% SDS-PAGE gel and subsequently transferred onto PVDF membranes (ISEQ00010, Merck Millipore, Germany). Then these membranes were blocked for 2 h in 5% skim milk. Following this, the membranes were incubated with primary antibodies overnight at 4°C and then with secondary antibodies for 1 h. Protein expression was detected using a western blot enhanced chemiluminescence substrate (K22020, Abbkine, Beijing, China), and the results were compared using the ImageJ software (Bio Rad, Hercules, CA, USA). The working dilutions of the antibodies against the following proteins were prepared: RORC(1:1000), IDO1(1:1000), Foxp3(1:2000) and GAPDH(1:10000).

2.7 Reverse Transcription-Quantitative PCR (RT-qPCR)

The spleen and colon tissues were subjected to RNA extraction using Trizol reagent (R404-01, Vazyme, Nanjing, Jiangsu, China). Subsequently, the RNA was reverse transcribed into cDNA using the PrimeScript RT reagent kit (TKB-RR047A, Takara, Kyoto, Japan). qPCR was performed using the ABI 7500 Fast PCR system with a SYBR Select Master Mix (TKB-RR420A, Takara, Kyoto, Japan)). The primer sequences are listed in Table 1. The reaction conditions of qPCR were as follows: 37°C for 15 min, followed by 85°C for 5 s. The relative quantification of the target genes was carried out by the $2^{-\Delta\Delta C_t}$ method, and GAPDH was used as the internal control.

Table 1
Real time PCR primer sequence

Gene	Sequence(5'-3')
ROR γ t	F 5'-GCAAATACGGTGGTGTGGAG-3' R 5'-GGACGGTTGGCATTGATGAG-3'
FOXP3	F 5'-TTACTCGCATGTTGCGCTAC-3' R 5'-AATTCATCTACGGTCCACACTG-3'
IDO1	F 5'-CCAGTGCAGTAGAGCGTCAA-3' R 5'-CGTGTCTGGGTCCACAAAGT-3'
IL- 17	F 5'-CCACGTCACCCTGGACTCTC-3' R 5'-CTCCGCATTGACACAGCG-3'
IFN- γ	F 5'-AAGACAATCAGGCCATCAGC-3' R 5'-CTGGACCTGTGGGTTGTTGA-3'
IL-10	F 5'-CTTACTGACTGGCATGAGGATCA-3' R 5'-GCAGCTCTAGGAGCATGTGG-3'
TGF- β	F 5'-TCTAGAATGCCGCCCTCGGGGCT-3' R 5'-TTCGAATCAGCTGCACTTGCAGGAGC-3'
IL-21	F 5'-CGCCTCCTGATTAGACTTCGT-3' R 5'-GGCTTGAGTTTGGCCTTCTG-3'
IL-22	F 5'-GCAGATAACAACACAGATCTCC-3' R 5'-GTCTTCCAGGGTGAAGTTGAG-3'
IL-25	F 5'-GACAGGGACTTGAATCGGGT-3' R 5'-TGTGGTAAAGTGGGACGGAG-3'
TNF- α	F 5'-GCCGATGGGTTGTACCTTGT-3' R 5'-TCTTGACGGCAGAGAGGAGG-3'
IL-1 β	F 5'-GAAATGCCACCTTTTGACAGTG-3' R 5'-TGGATGCTCTCATCAGGACAG-3'
GADPH	F 5'-TGGAAAGCTGTGGCGTGATG-3' R 5'-TACTTGGCAGGTTTCTCCAGG-3'

3. Statistical Analysis

The data were graphed by Prism Graphpad 8.0 and analyzed with SPSS 26.0 software. All data using the normal distribution and variance homogeneity test, t test is used between the two groups. The body weight, fecal occult blood score and DAI index of mice were analyzed by repeated measures analysis of variance. Data are presented as the mean $\pm$ SD, and $P < 0.05$ was considered statistically significant.

4. Results

1. QFG inhibits the development of colitis in DSS-induced UC mice.

During the experiment, it was observed that the weight of mice in the Control group increased steadily, with no fecal occult blood and diarrhea, and the DAI index was within normal range. These aforementioned symptoms began to appear about 3 days after the administration of DSS drinking water in both the Model group and QFG group. Following the cessation of DSS consumption, the body weight of mice in both the Model group and QFG group exhibited a gradual increase, while fecal occult blood and the DAI index decreased gradually. Compared with the Model group, it was observed that the weight increase of mice in the QFG group was more obvious, and the fecal blood intake and DAI index decreased more significantly, as shown in Figure 1A. In addition, the colons of mice in the Control group exhibited smooth, complete and uniformly colored characteristics. Conversely, the Model group displayed colonic congestion, edema, uneven color, localized dark purple, ulcers or erosions in multiple regions, accompanied by a blurred vascular network and a significantly reduced average length ($P < 0.05$). However, the QFG group demonstrated significant improvements in the aforementioned conditions compared to the Model group ($P < 0.05$), as shown in Figure 1B. The results of HE staining showed that the Control group exhibited an intact colonic mucosa with regular cell morphology and arrangement, orderly colonic villi, and no inflammatory cell infiltration. Conversely, the Model group displayed a damaged colonic villi structure, surface cell necrosis and exudation, cell deformation, and widened crypt space (as shown by the arrow). Compared with the Model group, the above conditions in the QFG group were significantly improved. These results collectively validate the efficacy of QFG in mitigating the weight loss, fecal occult blood and DAI index in DSS-induced UC mice, as well as alleviating the shortening of colon length and ameliorating the colonic histopathological changes caused by DSS in UC mice, as shown in Figure 1C.

2. QFG reduces the expression of IL-1 β , TNF- α , IFN- γ and IL-6 in DSS-induced UC mice.

The cytokines IL-1 β , TNF- α , IFN- γ , and IL-6 play significant roles in the tissue-mediated inflammatory response, serving as indicators of the body's inflammatory reaction. To assess the expression levels of these cytokines in spleen, colon and serum, RT-qPCR and Bio-plex technique was employed respectively. As shown in Figure 2A and B, compared with the Control group, the mRNA levels of cytokines IL-1 β , TNF- α and IFN- γ in the Model group were significantly increased in spleen and colon tissue ($P < 0.05$). Conversely,

the QFG group demonstrated a significant decrease ($P<0.05$) compared to the Model group ($P<0.05$). Comparable findings were observed in the serum (Figure 2C). These results indicate that the induction of UC in mice by DSS leads to an elevation in inflammatory factors, while the administration of QFG demonstrates the potential to mitigate inflammation by modulating the levels of these cytokines.

3. QFG decreases the expression of IDO1 in DSS-induced UC mice.

IDO1 plays a crucial role in maintaining immune homeostasis within the intestines by restraining inflammatory reactions and protecting the integrity of epithelial cells, and serves as a pivotal prognostic indicator for inflammatory conditions. In order to assess alterations in colon tissue and serum IDO1 levels, ELISA RT-qPCR Western Blot and IHC were used to detect the expression of IDO1. As shown in Figure 3A-C, IDO1 was increased in the Model group compared with the Control group in serum ($P<0.05$), after the administration of QFG, IDO1 was decreased ($P<0.05$). IDO1 mRNA and protein expression in colon tissue were significantly increased in the Model group compared with the Control group ($P<0.05$). Conversely, the QFG group demonstrated a significant decrease compared with the Model group ($P<0.05$). Comparable findings were observed in the IHC(Figure 3D). It was confirmed that DSS administration resulted in elevated IDO1 expression in both the serum and colon tissue, while QFG treatment led to a reduction in IDO1 expression in both the serum and colon tissue.

4. QFG decreases the expression of Th17-related cytokines and increases the expression of Treg-related cytokines in DSS-induced UC mice.

Based on the aforementioned experimental findings, it is evident that QFG exhibits a protective influence on DSS-induced UC mice. In order to further investigate its underlying mechanism, we detected the levels of Th17 and Treg-related cytokines. As shown in Figure 4A, compared with the Control group, Th17-related cytokines (IL-17, IL-21, IL-22, IL-25) were significantly increased in the Model group. And Treg-related cytokines (IL-10 and TGF- β) were slightly increased, with statistical differences. ($P<0.05$). Following QFG treatment, there was a significant decrease in Th17-related cytokines (IL-17, IL-21, IL-22, IL-25) compared to the Model group ($P<0.05$), while Treg-related cytokines (IL-10, TGF- β) exhibited a significant increase ($P<0.05$).

RT-qPCR was used to detect the mRNA expression levels of Th17 and Treg-related cytokines in spleen and colon tissues of mice, and the expression results were consistent with those in serum, as shown in Figure 4B-C. These results indicated that DSS administration induced a significant increase of Th17-related cytokines (IL-17, IL-21, IL-22, IL-25) and a increase in Treg-related cytokines (IL-10, TGF- β) in UC mice. After QFG treatment, there was a reduction in the expression of Th17-related cytokines (IL-17, IL-21, IL-22, IL-25), and an increase in the expression of Treg-related cytokines (IL-10, TGF- β).

5.QFG decreases RORyt and increases Foxp3 expression in DSS-induced UC mice.

To further investigate the mechanism of QFG on Th17 and Treg-related cytokines in UC mice, RT-qPCR, IHC and Western blot were used to detect the expression of transcription factors RORyt and Foxp3 in

spleen and colon tissues of mice. As shown in Figure 5A-E, compared with the Control group, the expression of ROR γ t mRNA and protein in spleen tissues of the Model group was significantly increased ($P<0.05$), and the expression of Foxp3 protein was also increased although no difference was observed in Foxp3 mRNA expression. Compared with the Model group, ROR γ t in the QFG group was significantly decreased, and Foxp3 in the QFG group was significantly increased ($P<0.05$). These results indicated that QFG could decrease the expression of ROR γ t and increase the expression of Foxp3.

5. Discussion

The immune imbalance of Th17/Treg is one of the important contributing factors for promoting inflammatory response and inducing autoimmune diseases. The IL-6-JAK-STAT3 pathway upregulates the expression of transcription factors ROR γ t and ROR α , thereby facilitating the differentiation of Th17 cells. These Th17 cells subsequently release characteristic cytokines, including IL-17A, IL-17F, IL-21, IL-22, and IL-23^[18]. IL-17 plays a crucial role in the initiation of inflammation by stimulating the synthesis of IL-6 and IL-8 via the mitogen-activated protein kinase (MAPK) pathway, thereby promoting the recruitment of neutrophils to the site of inflammation and triggering the proliferation of T cells. Additionally, IL-17 also promotes the expression of certain pro-inflammatory molecules such as inducible nitric oxide synthase (iNOS) and IL-1 β ^[19]. Treg cells possess immunomodulatory capabilities, enabling them to suppress immune responses triggered by diverse antigens and promote immune tolerance. In the presence of inflammation, activation of dendritic cells, monocytes, and macrophages prompts the rapid migration of Treg cells to the inflammatory site, effectively impeding the initiation and progression of inflammation at the site, thereby preserving immune homeostasis.

When the gut is stimulated to damage epithelial cells, inflammatory factors stimulate dendritic cells to produce IL-6 and up-regulate transcription factor ROR γ t, thus promoting Th17 cell differentiation and producing IL-17^[20]. At the same time, it also promotes the recruitment of neutrophils into the intestine, stimulates fibroblasts, macrophages, etc., and then induces the production of a variety of pro-inflammatory cytokines, resulting in intestinal inflammation. Continuous inflammatory stimulation leads to a decrease in the number of Treg cells and a weakening of their function. At this time, CD4⁺ T cells tend to differentiate into Th17 cells, and the pro-inflammatory factors secreted by Th17 cells rapidly increase, leading to further aggravation of intestinal mucosal inflammation^[21]. The secretion of IL-10 by Treg cells has the ability to impede the synthesis of pro-inflammatory cytokines, including IL-1 β , IL-6, and TNF- α , thereby exerting an inhibitory influence on T-cell-mediated immune responses^[22]. The functional opposition between Th17 and Treg cells is intricately linked to the development of chronic inflammatory responses, malignant tumors, and various autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus, and inflammatory bowel disease^[23–27]. UC is a chronic non-specific inflammatory disease of the intestine, wherein the dysfunction of intestinal mucosal immune system plays a crucial role in the pathogenesis of UC. This dysfunction encompasses abnormalities in both innate and adaptive immunity within the intestinal mucosa. At present, studies on congenital immunity mainly include the integrity of intestinal epithelial barrier and autophagy. Conversely, studies pertaining to adaptive

immunity primarily concentrate on the inflammatory response mediated by B cells and T cells within the lamina propria of the intestinal mucosa^[28, 29]. Th17 and Treg cells are integral components of the intestinal mucosal immune system, exerting significant influence. When the gut is stimulated to damage epithelial cells, inflammatory factors stimulate dendritic cells to produce IL-6 and up-regulate the expression of transcription factor ROR γ t, thus facilitating the differentiation of Th17 cells and producing IL-17^[30]. Simultaneously, it has the potential to facilitate the recruitment of neutrophils to the intestinal region, activate fibroblasts, macrophages, and other immune cells, as well as trigger the synthesis of various pro-inflammatory cytokines, culminating in the onset of intestinal inflammation. Prolonged exposure to inflammatory stimuli results in a reduction in the population of Treg cells and a decline in their regulatory function. At this time, CD4⁺ T cells exhibit a propensity to differentiate into Th17 cells, which in turn secrete heightened levels of pro-inflammatory mediators, thereby exacerbating the inflammatory response within the intestinal mucosa^[21]. The gut's innate immune response facilitates the generation of Treg cells while restraining the excessive inflammatory reaction of effector T cells. Consequently, preserving the equilibrium between Th17 and Treg cells emerges as a crucial strategy in averting the onset of UC.

The colitis model of DSS induced intestinal epithelial injury is one of the most widely used UC models^[31]. In this study, it was found that during the construction of UC model, QFG can inhibit weight loss in UC mice, relieve diarrhea, fecal occult blood, and DAI index. It alleviated the shortening of colon length induced by DSS and alleviated the pathological injury of colon tissue. These results suggest that QFG can improve the overall well-being of mice and effectively resist the intestinal damage induced by DSS in UC mice.

Given that DSS has been observed to induce notable colon inflammation in mice with UC, it is noteworthy that the immune cells infiltrating the inflamed tissues release pro-inflammatory cytokines such as IL-1 β , TNF- α , IFN- γ , IL-6, among others. It has been established that the expression levels of these factors exhibit a positive correlation with the severity of UC, thus rendering them suitable indicators for assessing the severity of this condition^[32]. Therefore, in this study, we detected the levels of IL-1 β , TNF- α , IFN- γ , IL-6 and other cytokines in serum, spleen and colon tissues of mice, so as to determine whether QFG can inhibit colon inflammation in UC mice. In addition, during the occurrence and development of UC, IDO1 is also significantly elevated. This enzyme is the first rate-limiting catalytic enzyme of tryptophan along the kynuridine metabolic pathway and is widely expressed throughout the body.

Furthermore, in the context of ulcerative colitis (UC), there is a notable increase in the expression of indoleamine 2,3-dioxygenase 1 (IDO1). This particular enzyme serves as the primary regulatory catalyst within the kynuridine metabolic pathway for tryptophan, and exhibits widespread distribution across various bodily tissues. Studies have confirmed that augmented levels of inflammatory cytokines such as IL-1 β , IFN- γ and TNF- α , elicit a notable upregulation of IDO1^[33, 34], especially in colon tissue. In addition, increased expression of IDO1 can promote the occurrence and development of inflammation through mediating TLR4/MyD88/NF- κ B signaling^[35, 36], which is an important inflammatory signal in the

development of UC ^[37] and an important indicator to evaluate the prognosis of UC inflammation. In this study, the results showed that the expression of cytokines such as IL-1 β , TNF- α , IFN- γ , IL-6 and IDO1 in UC mice was significantly increased compared with that in the Control group, indicating that the DSS induced UC model had a significant inflammatory response. However, QFG decreased the expression of IL-1 β , TNF- α , IFN- γ , IL-6, IDO1 in mice, indicating that QFG inhibited colon inflammation in UC mice.

In order to explore whether the mechanism of QFG inhibiting colon inflammation in UC mice is related to the regulation of Th17/Treg cell balance, we examined the changes of cytokines related to Th17 and Treg cells. Inflammation stimulates the body. First, Treg cells play a role in intestinal innate immunity and accelerate Treg cell differentiation to inhibit T cell-mediated inflammatory response. The results of this study showed that after DSS administration, the cytokines associated with Treg cells in mice were slightly increased, which may be related to the innate immunity of Treg cells. With the continuous stimulation of inflammation, the number of Treg cells decreased and their function weakened, while Th17 cells took advantage of differentiation and secreted pro-inflammatory factors rapidly increased, leading to further aggravation of intestinal mucosal inflammation. This is consistent with the results of this study, which showed that after DSS administration, the cytokines related to Th17 cells in mice were significantly increased, while the cytokines related to Treg cells, TGF- β and IL-10, were slightly increased. After QFG treatment, Th17-related cytokines IL-17, IL-21, IL-22 and IL-25 were significantly decreased, while Treg-related cytokines TGF- β and IL-10 were significantly increased, restoring the balance of Th17/Treg cells. This may be related to QFG inhibiting the differentiation of Th17 cells and promoting the differentiation of Treg cells. Therefore, we further examined the changes of transcription factor ROR γ t in Th17 cells and Foxp3 in Treg cells. The results showed that ROR γ t was significantly increased and Foxp3 was slightly increased after DSS treatment. After QFG treatment, ROR γ t was significantly decreased and Foxp3 was significantly increased, which was consistent with the changes of cytokines related to Th17 and Treg cells. This suggests that ROR γ t and Foxp3 affect the changes of Th17 and Treg cell related cytokines. Therefore, QFG may inhibit the transcription of ROR γ t and thus inhibit the differentiation of Th17 cells, and promote the up-regulation of transcription factor Foxp3 to increase the differentiation of Treg cells and maintain the Th17/Treg cell balance. Thus, the intestinal inflammatory response of UC mice induced by DSS was inhibited.

6. Conclusion

In conclusion, the findings suggest that QFG may have a beneficial effect on various aspects of colitis development in UC mice induced by DSS. These include mitigating weight loss, fecal occult blood, DAI, colon length shortening, and pathological injury of the colon. Additionally, QFG appears to reduce the expression of pro-inflammatory cytokines IL-1 β , TNF- α , IFN- γ , and IL-6, as well as the expression of IDO1. The underlying mechanism may involve the inhibition of Th17 cell differentiation, promotion of Treg cell differentiation, and maintenance of Th17/Treg cell balance through the regulation of transcription factors ROR γ t and Foxp3. These findings provide a preliminary experimental basis for potential clinical applications.

Abbreviations

English abbreviation	Full name
CACC	Colitis-Associated Colon Cancer
DAI	Disease activity index
DSS	Dextra sulfate sodium
ELISA	Enzyme-linked immuno sorbent assay
FOB	Fecal occult blood
Foxp3	Forked head/wing helix transcription factor 3
HE	Hematoxylin and eosin
IBD	Inflammatory bowel diseases
IDO1	Indoleamine 2,3-dioxygenase 1
IFN- γ	Interferon- γ
IHC	Immunohistochemical staining
IL-1 β	Interleukin-1 β
IL-6	Interleukin-6
IL-10	Interleukin-10
IL-17	Interleukin-17
IL-21	Interleukin-21
IL-22	Interleukin-22
IL-23	Interleukin-23
IL-25	Interleukin-25
iNOS	Inducible nitric oxide synthase
JAK-STAT3	Janus Kinase-Signal transducers and activators of transcription 3
MDA	malondialdehyde
MPO	peroxidase
MyD88	Myeloid differentiation factor 88
NF- κ B	Nuclear factor kappa-B
OFR	oxygen free radical
PBS	Phosphate buffer saline
QFG	Qingjie Fuzheng Granules

RORα	(Retinoid-related orphan receptor-α)
RORγt	Retinoic acid-associated orphan receptor γt
RT-qPCR	Real Time Quantitative PCR
TGF-β	Transforming growth factor-β
Th 17	Helper T cells 17
TLR4	Toll-like receptor 4
TNBS	Trinitrobenzenesulfonic acid
TNF-α	Tumor necrosis factor-α
Treg	Regulatory T cells
UC	Ulcerative colitis
WB	Western Blot

Declarations

Ethics approval and consent to participate All animal experiments were conducted in accordance with the national guidelines for the humane treatment of animals and were approved by the Ethics Committee of Fujian University of Traditional Chinese Medicine (FJTCMIACUC 2022039).

Consent for publication

Availability of data and materials The datasets used and/or analyzed during the current study will be available from the corresponding author upon reasonable request.

Conflicts of interest: Authors declare no conflicts of interest.

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Authors' contributions: H Z and H L conducted animal experiments and data statistical analysis, and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

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Image

Image1 is available in the Supplementary Files section.

Figures

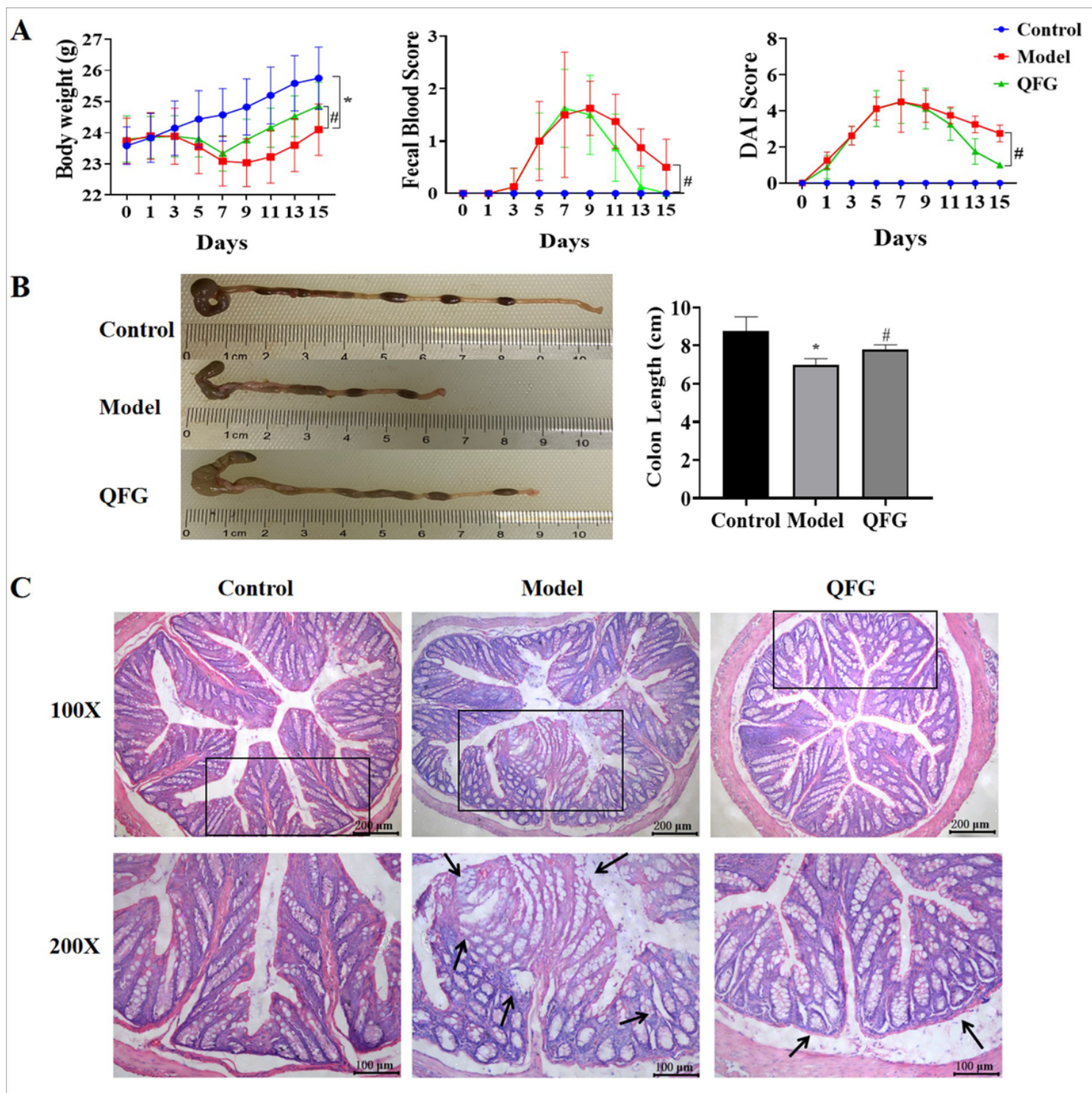


Figure 1

QFG inhibit the development of colitis in DSS induced UC mice. (A) Effects of QFG on the body weight, Fecal occult blood and DAI index in DSS-induced UC mice. (B) Effects of QFG on colon length in DSS-induced UC mice. (C) Effect of QFG on colon histopathology in DSS-induced UC mice (100 $\times$ and 200 $\times$). Note: Model vs Control group, * P <0.05; QFG vs the Model group, # P <0.05.

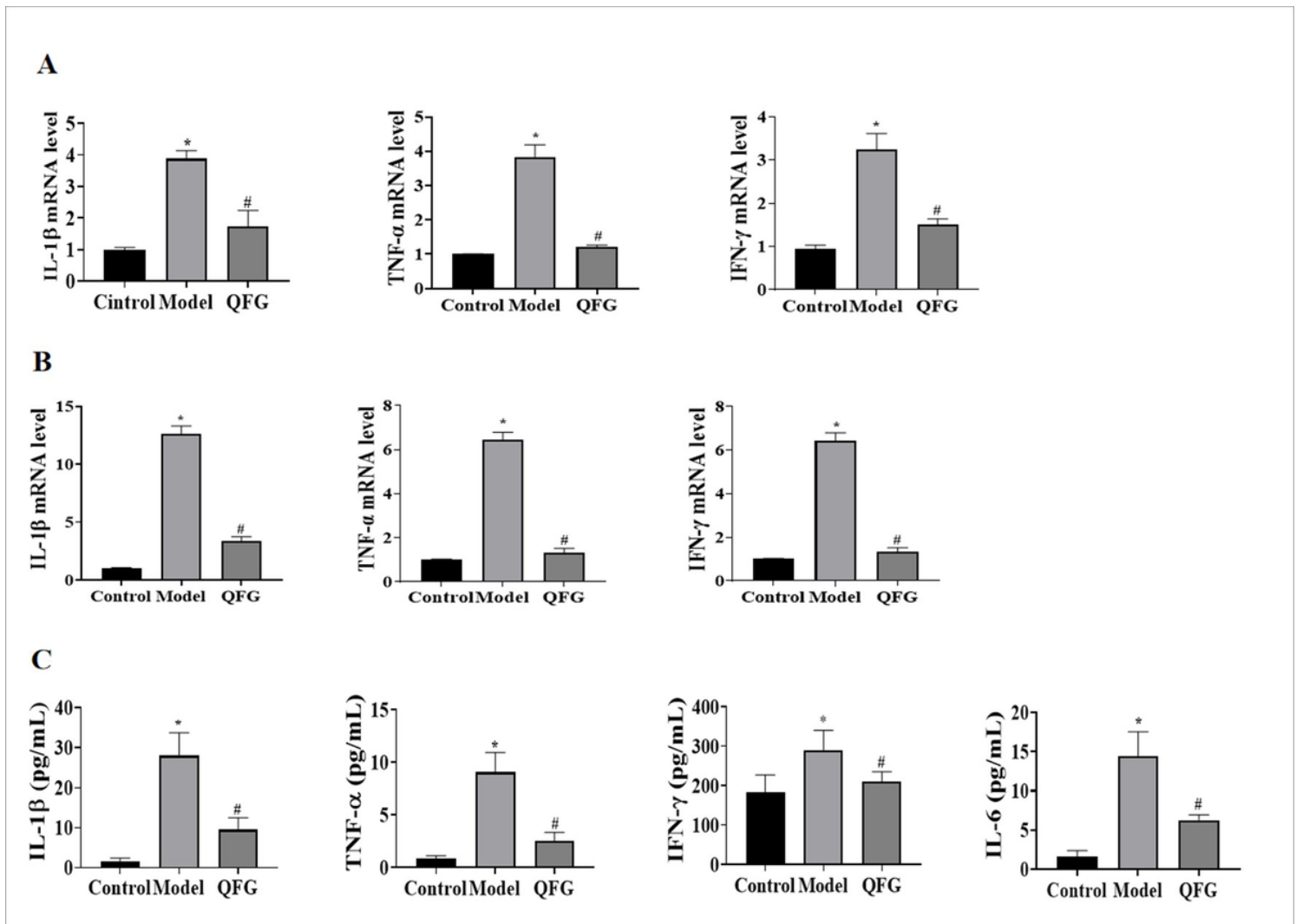


Figure 2

QFG decreased the expression of related inflammatory factors in serum, spleen and colon in DSS-induced UC mice. (A) Effects of QFG on cytokines IL-1 β , TNF- α and IFN- γ in spleen tissue were detected by RT-qPCR. (B) Effects of QFG on cytokines IL-1 β , TNF- α and IFN- γ in colon tissue were detected by RT-qPCR. (C) Effects of QFG on cytokines IL-1 β , TNF- α , IFN- γ and IL-6 in serum were detected by Bio-plex. Note: Model vs Control group, * P <0.05; QFG vs the Model group, # P <0.05.

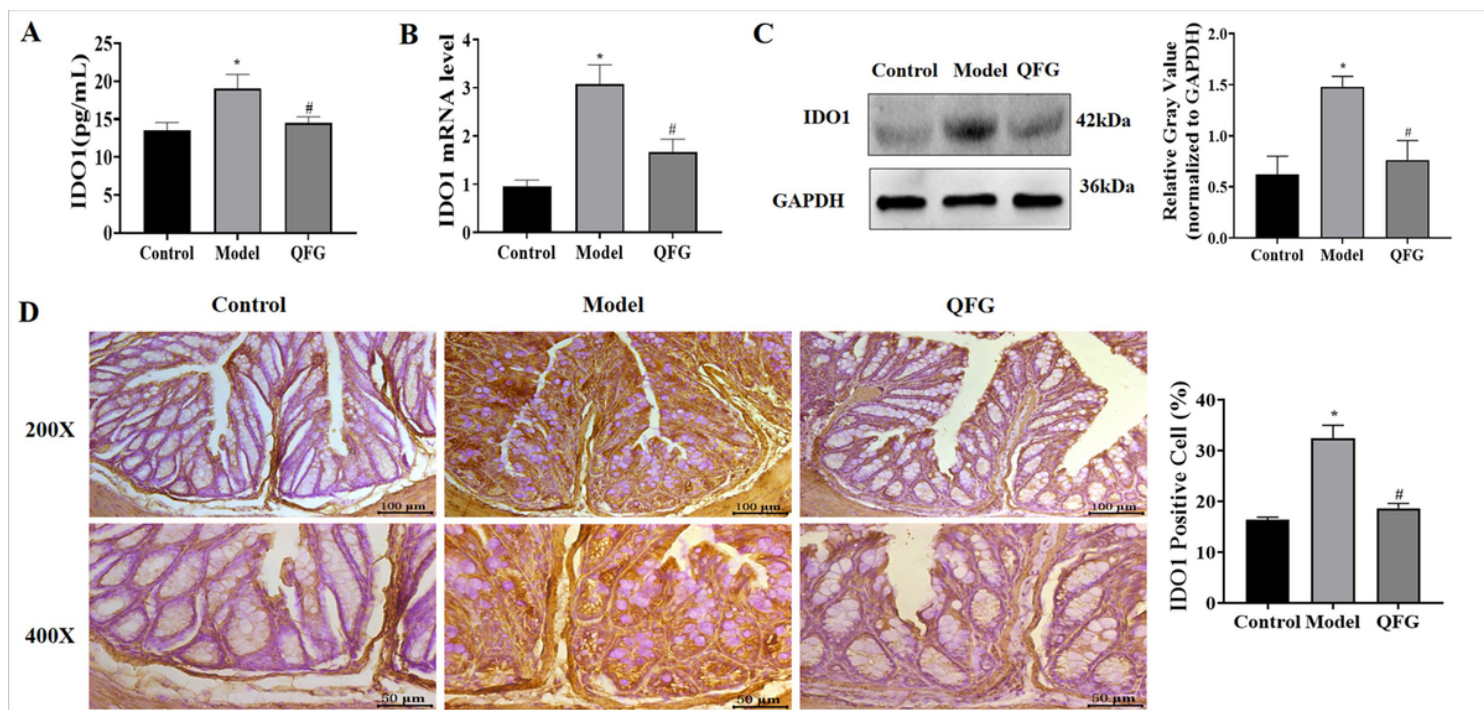


Figure 3

QFG decreased IDO1 expression in serum and colon tissue in DSS-induced UC mice. (A) Effect of QFG on IDO1 levels in serum (B) Effect of QFG on IDO1 mRNA in colon tissue. (C) Effect of QFG on IDO1 protein in colon tissue. (D) IHC analysis was performed to determine the IDO1 expression of tumors in each group. Note: Model vs Control group, * $P < 0.05$; QFG vs the Model group, # $P < 0.05$.

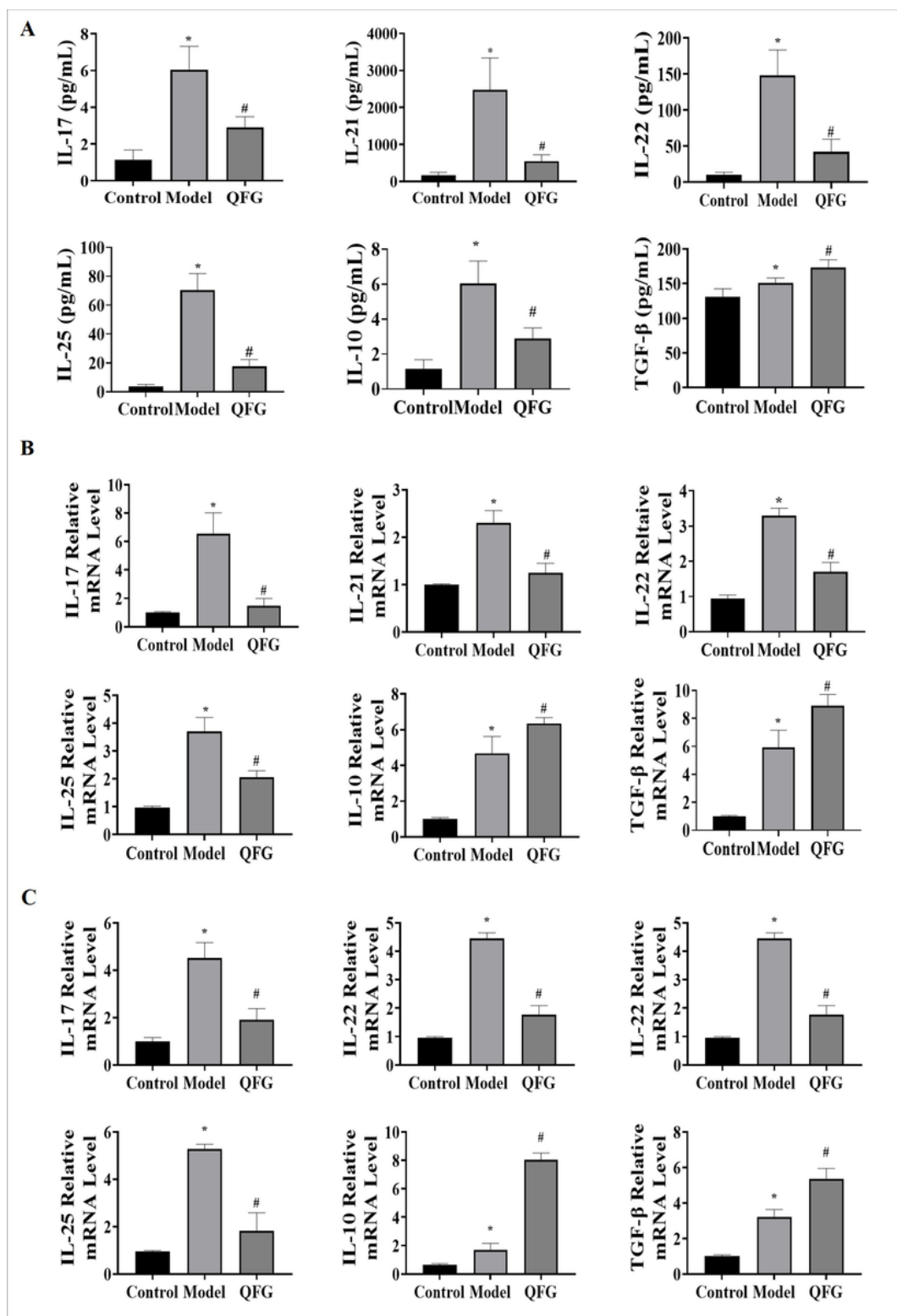


Figure 4

QFG decreases the expression of Th17-related cytokines and increases the expression of Treg-related cytokines in serum, spleen and colon. (A) Effects of QFG on Th17- and Treg-related cytokines IL-17, IL-21, IL-22, IL-25, IL-10 and TGF-β in serum of DSS-induced UC mice. (B) Effects of QFG on Th17- and Treg-related cytokines IL-17, IL-21, IL-22, IL-25, IL-10 and TGF-β in spleen tissue of DSS-induced UC mice. (C) Effects of QFG on Th17- and Treg-related cytokines IL-17, IL-21, IL-22, IL-25, IL-10 and TGF-β in colon

tissue of DSS-induced UC mice. Note: Compared with the Control group, *P<0.05; Compared with the Model group, #P<0.05.

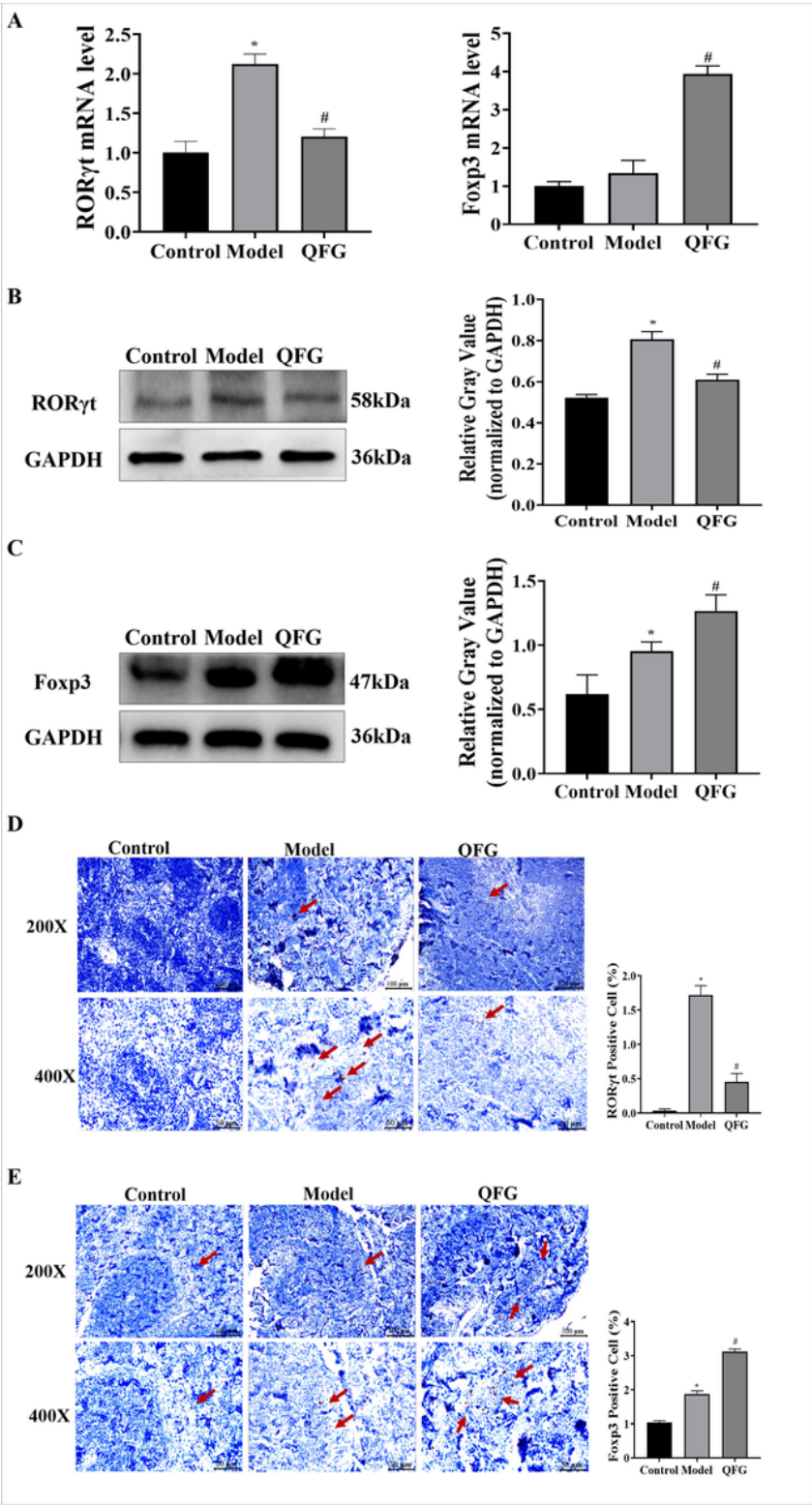


Figure 5

QFG decreases RORγt and increases Foxp3 expression in spleen tissue of DSS-induced UC mice. (A) Effect of QFG on mRNA of RORγt and Foxp3 in spleen tissue of DSS induced UC mice. (B) Effect of QFG

on RORyt proteins in spleen tissue of DSS induced UC mice. (C) Effect of QFG on FoxP3 proteins in spleen tissue of DSS induced UC mice. (D) The expression of RORyt in spleen tissue of DSS induced UC mice induced by QFG was analyzed by IHC. (E) The expression of FoxP3 in spleen tissue of DSS induced UC mice induced by QFG was analyzed by IHC Note: Compared with the Control group, * $P<0.05$; Compared with the Model group, # $P<0.05$.

Supplementary Files

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